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EXAMINER

WESSENDORF, TERESA D

ART UNIT

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1639

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of the linker species of 10 amino acids length in the reply filed on 4/7/08 is acknowledged. The traversal is on the ground(s) that a search for each allegedly distinct species is not unduly burdensome and, indeed, has already been conducted. This case has a lengthy prosecution history, including an entire Appeal of claims 5, 6 and 20 in their current form. All prosecution and the appeal were conducted without any need for an election of species. This is not found persuasive because the prior prosecution did not provide a limitation as to the peptide length of the linker as presently claim in the newly added claim 21. Thus, a search has not been conducted on the newly presented claim since this limitation has not been presented in the prior lengthy prosecution. Indeed it would be a burdensome search to examine a peptide length between 8 and 25 long. A prior art reference anticipating an 8-residue peptide would not render obvious a 25-residue peptide linker especially for a non-naturally occurring linker.

The requirement is still deemed proper and is therefore made FINAL.

Status of Claims

Claims 5-6 and 20-21 (with respect to the elected species of 10-residue length) are pending and under examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 5 and 21 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The specification fails to provide an adequate written description of a fusion protein linked by e.g., a 10-residue long non-naturally occurring peptide linkers. The specification does not define the amino acid residues contained in the non-naturally occurring peptide linkers. One cannot ascertain as to

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the different kind/position of non-naturally occurring amino acids that are contained within the claimed non-naturally occurring peptide. The specification at e.g., paragraph beginning on page 15, line 16 provides a list of naturally occurring peptide linkers (based on the naturally occurring amino acid residues present in the linkers):

T G E K P: (SEQ ID NO:3) (Liu et al., 1997, supra.); (G4S)_n (SEQ ID NO:4) (Kim et al., PNAS 93, 1156-1160 (1996.)); GGRRGGGS (SEQ ID NO:5); LRQRDGERP (SEQ ID NO:6); LRQKDGGGSERP (SEQ ID NO:7); LRQKD(G3S)₂ ERP (SEQ ID NO:8). Alternatively, flexible linkers can be rationally designed using computer program capable of modeling both DNA-binding sites and the peptides themselves or by phage display methods. In a further variation, noncovalent linkage can be achieved by fusing two zinc finger proteins with domains promoting heterodimer formation of the two zinc finger proteins. For example, one zinc finger protein can be fused with fos and the other with jun (see Barbas et al., WO 95/119431).

Thus, the specification does not disclose, at the time of filing, what constitutes a non-naturally occurring peptide linkers of 10 length residues. Assuming arguendo that the non-naturally occurring linkers are the ones synthetically produced, however, from the above given lists, no description of the elected 10-residue length peptide is adequately provided by the listing, at the time of filing. There is no description as to the kind of amino acids contained in a 10-residue length peptide linker. It does

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not describe the residue(s) responsible for linking the two or more fusion proteins, whether each of the proteins each contain 10 residues and how linkage in the protein occurs. There is no guidance or direction as to how one can make the claimed 10-residue non-naturally occurring linker such that it can link the zinc finger complex, as claimed.

To satisfy a written description requirement for a claimed genus a sufficient description of a representative number of species by actual reduction to practice or by disclosure of relevant, identifying characteristics, i.e., **structure** or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. A listing of every possible linker does not constitute a written description of every species in a genus. It would not "reasonably lead" those skilled in the art to any particular species. In re *Ruschig*, 379 F.2d 990, 995, 154 USPQ 118, 123 (CCPA 1967)

This is especially true since there is not a single naturally occurring 10-residue length peptide (from which the non-naturally occurring peptide linker can be based upon) described in the specification at the time of filing.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

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(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 5-6 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pomerantz in view of Krylov [or Marmostein] for the reasons of record based on the Board's decision and reiterated below.

The Pomerantz publication has been described for its disclosure of a zinc finger fused to the naturally occurring dimerization domain extracted from the GAL4 protein. Pomerantz's fusion protein differs from the fusion protein contained in the zinc finger complex of claim 5 by having a naturally occurring dimerization domain, instead Pomerantz points the skilled artisan directly to prior art publications that teach modified dimerization domains. Such domains are non-naturally occurring and "join each other by specific binding," meeting the requirements of the claimed "peptide linkers." See claim 5. In particular, reference 19 (hereinafter "Krylov"), cited by Pomerantz for its studies of the coiled-coil interaction motif, describes "protein design rules that can be used to modify leucine zipper-containing proteins to possess novel dimerization properties." Krylov, page 2850, column 1. "33 different leucine

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zipper proteins containing 27 different systematic combinations of amino acids" were produced. Id., page 2856, column 2 ("Discussion"). See also Fig. 1B for a list of exemplary "mutant proteins." Id., page 2850, column 2. The mutant proteins were mixed together under conditions which facilitated dimer formation. By measuring the stability of the dimers formed (id., page 2852-53, "Thermodynamic stability"), Krylov was able to demonstrate that certain modified dimers had increased stability and specificity as compared to the unmodified form. ("Novel heterologous interactions regulate dimerization specificity In the second mixing experiment, the stability of the heterodimer is calculated to be greater than the average of the two homodimer stabilities, thus favoring the formation of heterodimers." Id., page 2856, columns 1-2.) Thus, the element missing from Pomerantz - non-naturally occurring peptide linkers - is supplied by Krylov. The skilled worker would have had a reasonable expectation that Krylov's domains could be utilized to complex zinc fingers to which they are attached in view of Krylov's success in not only modifying their binding activity, but in making it stronger (i.e., more stable). Krylov also teaches dimerization domains having the same sequence, meeting the limitations of claim 6. See e.g., id., page 2856, column 1, describing homo- and heterodimers, where the homodimers have

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"the same sequence." Pomerantz describes dimers between ZFGD1 fusion protein, where each fusion contains the same zinc finger. Pomerantz, Abstract ("a dimeric zinc finger protein, ZFGDI"). This meets the requirements of claim 20. In sum, we find that Pomerantz and Krylov disclose all elements of the subject matter recited in claims 5, 6, and 20. For the reasons discussed above, the skilled worker would have considered these claims obvious in view of Pomerantz's express suggestion to combine its teaching with Krylov (i.e., reference 19), and Krylov's disclosure that would have led the skilled worker to reasonably expect that the combination would work.

Response to Arguments

Applicants argue that the citation in Pomerantz to Krylov does not cure the deficiency of Pomerantz because Krylov fails entirely to teach or suggest anything about zinc finger proteins complexed together via non-naturally occurring peptides fused to each of the zinc finger proteins. Applicants however recognized that Krylov discloses only that certain amino acid residues that are part of naturally occurring leucine zipper proteins can be mutated to modulate dimerization stability and specificity. Applicants state that clearly, Krylov's dimerization mutants are not complexes of zinc finger proteins as claimed.

In reply, it is not clear how Krylov's dimer mutants are not complexes of ZFP when Pomerantz clearly made reference to the linker mutant of Krylov to dimerize the ZFPs.

Applicants recognize that Krylov mutates residues in the context of a single naturally occurring protein, but argue that this reference does not teach or suggest complexes in which each component of the complex is a fusion of a zinc finger protein and a non-naturally occurring linker.

In response, attention is drawn to the disclosure of Krylov above which states:

The mutant proteins were mixed together under conditions which facilitated dimer formation.

Thus, the need to mix a dimer together would indicate that each of the dimer is in separate state and mixed to form a particular dimer.

Applicants state that Krylov teaches away from combining mutant leucine zipper dimerization domains with heterologous zinc finger domains because this reference clearly states that the whole leucine zipper protein is involved in the specificity of dimerization (Krylov, page 2859, left column):

Since the specificity of dimerization is distributed throughout the length of the leucine zipper, the potential for modulation of dimerization partners is

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great. Thus, Krylov teaches that the importance of overall interactions of the leucine zipper protein.

In response, applicants have taken out of context

Krylov's teachings at page 2859. The whole text of the above-quoted section states:

The large number of leucine zipper proteins in mammalian systems suggests that a **judicious** combination of attractive and repulsive interactions may be needed to design **specific dimerization partners**. Since the specificity of dimerization is distributed throughout the length of the leucine zipper, the potential for modulation of dimerization partners is great. Our design of leucine zipper partners that preferentially heterodimerize can be a useful biological tool to bring together different cellular proteins at specific locations in the cell. (Emphasis added).

See further Krylov's design of the motif throughout the article, at e.g., page 2849, the abstract and paragraph bridging col. 1 and col. 2:

In order to generate a repeating helical dimerization interface...results in a repeating structural unit of two helical turns or seven amino acids (a heptad repeat). (Emphasis added).

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 102

Claims 5 and 6 are rejected under 35 U.S.C. 102(e) as being anticipated by Barbas et al (USP 6242568) or Eisenberg et al (6453242).

Barbas et al discloses at e.g., col. 28, line 25 up to col. 29, line 51:

...Zinc finger proteins containing from about 2 to 20 zinc fingers Zif(2) to Zif(20), and preferably from about 2 to 12 zinc fingers, may be fused to the leucine zipper domains of the Jun/Fos proteins, prototypical members of the bZIP family of proteins (O'Shea, et al., Science, 254:539, 1991). Alternatively, zinc finger proteins can be fused to other proteins which are capable of forming heterodimers and contain **dimerization domains. Such proteins will be known to those of skill in the art.**

The Jun/Fos leucine zippers are described for illustrative purposes and preferentially form heterodimers and allow for the recognition of 12 to 72 base pairs. Henceforth, Jun/Fos refer to the leucine zipper domains of these proteins. Zinc finger proteins are fused to Jun, and independently to Fos by methods commonly used in the art to link proteins. Following purification, the Zif-Jun and Zif-Fos constructs (SEQ ID NOS: 33, 34 and 35, 36 respectively), the proteins are mixed to spontaneously form a Zif-Jun/Zif-Fos heterodimer. Alternatively, coexpression of the genes encoding these proteins results in the formation of Zif-Jun/Zif-Fos heterodimers in vivo. Fusion of the heterodimer with an N-terminal nuclear localization signal allows for targeting of expression to the nucleus (Calderon, et al, Cell, 41:499, 1982). Activation domains may also be incorporated into one or each of the leucine zipper fusion constructs to produce activators of transcription (Sadowski, et al., Gene, 118:137, 1992). These dimeric constructs then allow for specific activation or repression of transcription. **These heterodimeric Zif constructs are advantageous since they allow for recognition of palindromic sequences (if the fingers on both Jun and Fos recognize the same DNA/RNA sequence) or extended asymmetric**

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sequences (if the fingers on Jun and Fos recognize different DNA/RNA sequences).

As recognized by the Zif268-Fos/Zif268 Jun dimer (x is any number). The spacing between subsites is determined by the site of fusion of Zif with the Jun or Fos zipper domains and the length of the linker between the Zif and zipper domains. Subsite spacing is determined by a binding site selection method as is common to those skilled in the art (Thiesen, et al., Nucleic Acids Research, 18:3203, 1990). Example of the recognition of an extended asymmetric sequence is shown by Zif(C7).sub.6 -Jun/Zif-268-Fos dimer. This protein consists of 6 fingers of the C7 type (EXAMPLE 11) linked to Jun and three fingers of Zif268 linked to Fos, and **recognizes the extended sequence...** (All emphasis added.)

Response to Arguments

Applicants argue that Barbas fails to disclose non-naturally occurring linkers. But acknowledge that Barbas discloses instead only the naturally occurring Fos/Jun dimerization domains (col. 28, lines 27-30 of Barbas):

Zinc finger proteins containing from about 2 to 20 zinc fingers Zif(2) to Zif(2), and preferably from about 2 to 12 zinc fingers, may be fused to leucine zipper domains of the Jun/Fos proteins ...

In reply, attention is drawn to Barbas disclosure which made reference to Ladner(U.S. Pat. No. 5,223,409, incorporated by reference in its entirety), wherein Ladner discloses at e.g., col. 7, lines 15-21:

Ladner and Bird...suggest that single chain "pseudodimeric" repressors (DNA-binding proteins) may be prepared by mutating a putative linker peptide followed by in vivo selection that mutation and selection may be used to create

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a dictionary of recognition elements for use in the design of asymmetric repressors.

Furthermore, since the fusion variant of Barbas is synthetically/recombinantly made hence it follows that Fos/Jun which is a part of the fusion protein is a non-naturally occurring peptide being synthetically made as taught by Barbas. (Note the specification discloses broadly only a linker that is non-naturally occurring peptide but does not recite a single non-naturally occurring peptide but also the Jun/fos linker). Thus, when read in the light of the specification, the same linker is used by Barbas as claimed.

Applicants further argue that in terms of dimerization domains other Fos/Jun, Barbas does not teach non-naturally occurring peptides and, moreover, teaches that domains other than Fos/Jun are used to link a zinc finger protein to a different protein, which is unlike the claimed complexes of 2 zinc finger proteins (Barbas, col. 28, lines 31-34, emphasis added):

Alternatively, zinc finger proteins can be fused to other proteins which are capable of forming heterodimers and contain dimerization domains

In response, as applicants stated above, Barbas teaches in the **alternative** heterodimers but throughout the patent teaches a homodimer.

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Claims 5 and 6 are rejected under 35 U.S.C. 102(e) as being anticipated by Eisenberg et al (6453242) for reasons of record as reiterated below.

Eisenberg et al discloses at e.g., col. 9, line 48 up to col.10, line 60:

Zinc finger proteins are formed from zinc finger components. For example, zinc finger proteins can have one to thirty-seven fingers, commonly having 2, 3, 4, 5 or 6 fingers. **A zinc finger protein recognizes and binds to a target site (sometimes referred to as a target segment) that represents a relatively small subsequence within a target gene. Each component finger of a zinc finger protein can bind to a subsite within the target site. The subsite includes a triplet of three contiguous bases all on the same strand (sometimes referred to as the target strand). The subsite may or may not also include a fourth base on the opposite strand that is the complement of the base immediately 3' of the three contiguous bases on the target strand. In many zinc finger proteins, a zinc finger binds to its triplet subsite substantially independently of other fingers in the same zinc finger protein. Accordingly, the binding specificity of zinc finger protein containing multiple fingers is usually approximately the aggregate of the specificities of its component fingers. For example, if a zinc finger protein is formed from first, second and third fingers that individually bind to triplets XXX, YYY, and ZZZ, the binding specificity of the zinc finger protein is 3'XXX YYY ZZZ5'.**

The relative order of fingers in a zinc finger protein from N-terminal to C-terminal determines the relative order of triplets in the 3' to 5' direction in the target. For example, if a zinc finger protein comprises from N-terminal to C-terminal the first, second and third fingers mentioned above, then the zinc finger protein binds to the target segment 3'XXXYYYZZZ5'. If the zinc finger protein comprises the fingers in another order, for example, second finger, first finger, third finger, then the zinc finger protein binds to a target segment comprising a different permutation of triplets, in this example, 3'YYYXXXZZZ5'

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(see Berg & Shi, Science 271, 1081-1086 (1996)). The assessment of binding properties of a zinc finger protein as the aggregate of its component fingers is, however, only approximate, due to context-dependent interactions of multiple fingers binding in the same protein.

Two or more zinc finger proteins can be linked to have a target specificity that is the aggregate of that of the component zinc finger proteins (see e.g., Kim & Pabo, PNAS 95, 2812-2817 (1998)). For example, a first zinc finger protein having first, second and third component fingers that respectively bind to XXX, YYY and ZZZ can be linked to a second zinc finger protein having first, second and third component fingers with binding specificities, AAA, BBB and CCC. The binding specificity of the combined first and second proteins is thus 3'XXXYYYZZZ_AAABBBCCC5', where the underline indicates a short intervening region (typically 0-5 bases of any type). In this situation, the target site can be viewed as comprising two target segments separated by an intervening segment.

Linkage can be accomplished using any of the following peptide linkers. TGE KP (SEQ ID NO:2) (Liu et al., 1997, supra.); (G.sub.4 S).sub.n (SEQ ID NO:3) (Kim et al., PNAS 93, 1156-1160 (1996.)); GGRRGGGS (SEQ ID NO:4); LRQRDGERP (SEQ ID NO:5); LRQKDGGGSERP (SEQ ID NO:6) ; LRQKD(G.sub.3 S).sub.2 ERP (SEQ ID NO:7). Alternatively, flexible linkers can be rationally designed using computer program capable of modeling both DNA-binding sites and the peptides themselves or by phage display methods. In a farther variation, noncovalent linkage can be achieved by fusing two zinc finger proteins with domains promoting heterodimer formation of the two zinc finger proteins. For example, one zinc finger protein can be fused with fos and the other with jun..

Response to Arguments

Applicants state that Eisenberg describes linking of zinc finger proteins via a single peptide linker that joins the zinc fingers to each other. This single linker does not specifically bind to a second linker in order to join the zinc finger

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proteins together. Similarly, Eisenberg does not disclose complexes as set forth in claims 5 and 6.

In reply, attention is directed to the disclosure of Eisenberg above which states:

In a farther variation, noncovalent linkage can be achieved by fusing two zinc finger proteins with domains promoting *heterodimer formation of the two zinc finger proteins*. For example, **one zinc finger protein can be fused with fos and the other with jun...** (Emphasis added.)

Note further the teachings of Eisenberg as to the different short length peptides used as a linker e.g., the different Seq. ID. Nos. and the rational design of said linker using computer program.

Claim Rejections - 35 USC § 103

Claims 5, 6, 20 and newly added claim 21, are rejected under 35 U.S.C. 103(a) as being unpatentable over Eisenberg et al.

Eisenberg as discussed above discloses:

Linkage can be accomplished using any of the following peptide linkers. TGE KP (SEQ ID NO:2) (Liu et al., 1997, supra.); (G.sub.4 S)n (SEQ ID NO:3) (Kim et al., PNAS 93, 1156-1160 (1996.)); GGRGGGS (SEQ ID NO:4); LRQDGERP (SEQ ID NO:5); LRQKDGGGSERP (SEQ ID NO:6) ; LRQKD(G.sub.3 S).sub.2 ERP (SEQ ID NO:7). Alternatively, flexible linkers can be rationally designed using computer program capable of modeling both DNA-binding sites and the peptides themselves or by phage display methods.

Accordingly, the broad claimed ten-residue amino acid linker is included in the specific peptide linker having the specific structures of the amino acid range e.g., 5-15 residues taught by Eisenberg. It would be within the ordinary skill in the art to pick and chose the ten residues from the given range with a reasonable expectation of success as taught by Eisenberg above.

No claim is allowed.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to T. D.


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Wessendorf whose telephone number is (571) 272-0812. The examiner can normally be reached on Flexitime.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James Schultz can be reached on 571 272-0763. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/T. D. Wessendorf/
Primary Examiner, Art Unit 1639

<div>Application Number</div> <div></div>	Application/Control No.	Applicant(s)/Patent under Reexamination	
	09/636,243	WANG ET AL.	
	Examiner	Art Unit	
	T. D. Wessendorf	1639	